Extension-Trapping SNP Assay

Highly stringent annealing conditions (gDNA is biotinylated prior to assay) :

T7   zip   U4   T7   zip   U5

gDNA   G——————   ——————G——————

Immobilize and wash

Saturate beads with free biotin

Add biotin-ddNTP and Taq Pol

T7   zip   U4   ddNTP-(B)   T7   zip   U5   ddNTP-(B)
                   Taq   Taq
——————C-(B)   ——————A
      G   ——————G——————

Wash and Elute

T7   zip   U4   T7   zip   U5
——————C-(B)   ——————A

Immobilize and Wash

T7   zip   U4   Streptavidin coated
——————C   magnetic bead
        Cy3

Alternatively, this can be carried on to a
T7 transcription step yielding an RNA:

(RNA) —————— zip
                    U4

RNA is first hybridized to array
by zip code, then probed with the
fluorescent labeled PCR primers

PCR

T7   zip   U4
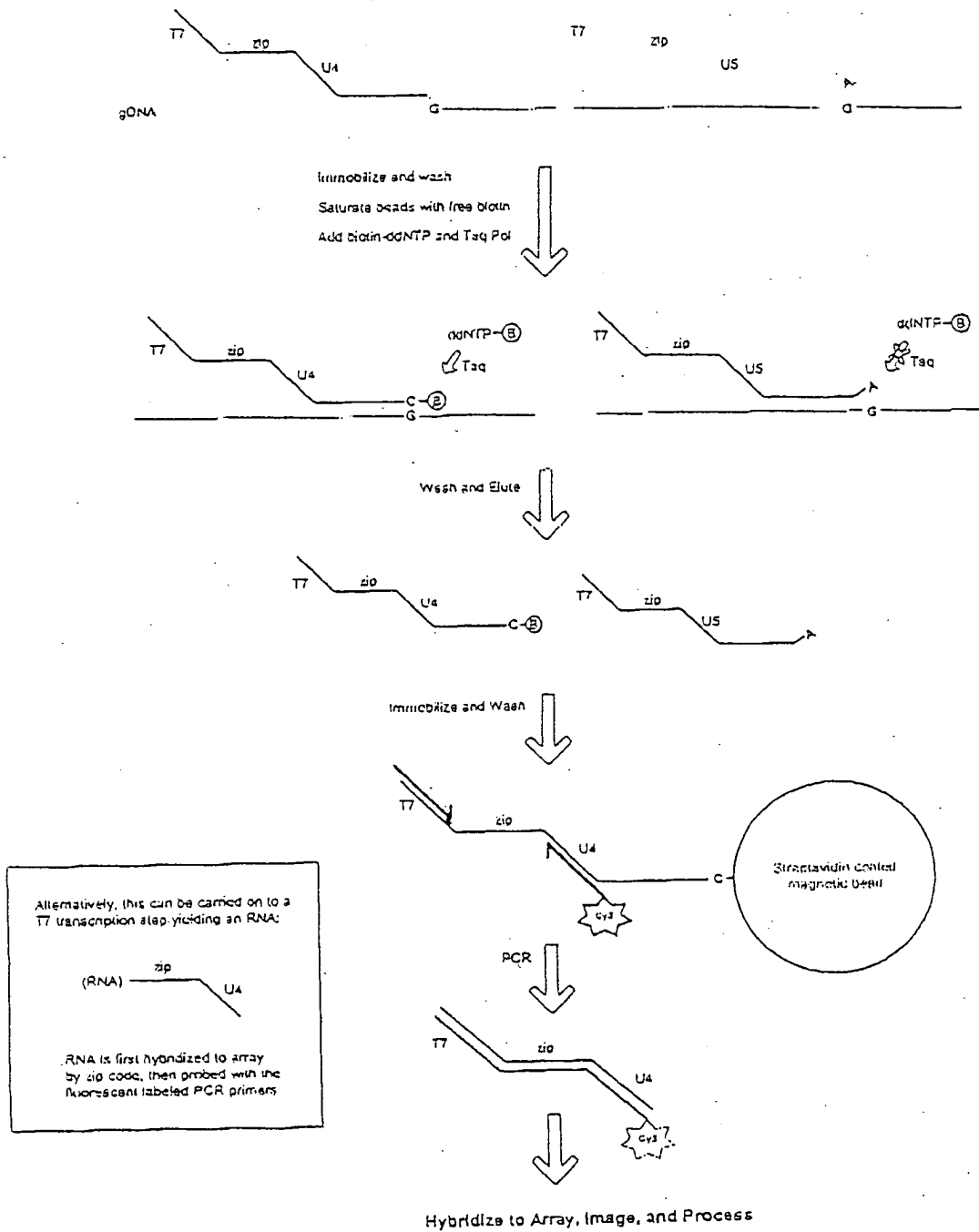   Cy3

Hybridize to Array, Image, and Process

FIGURE 1
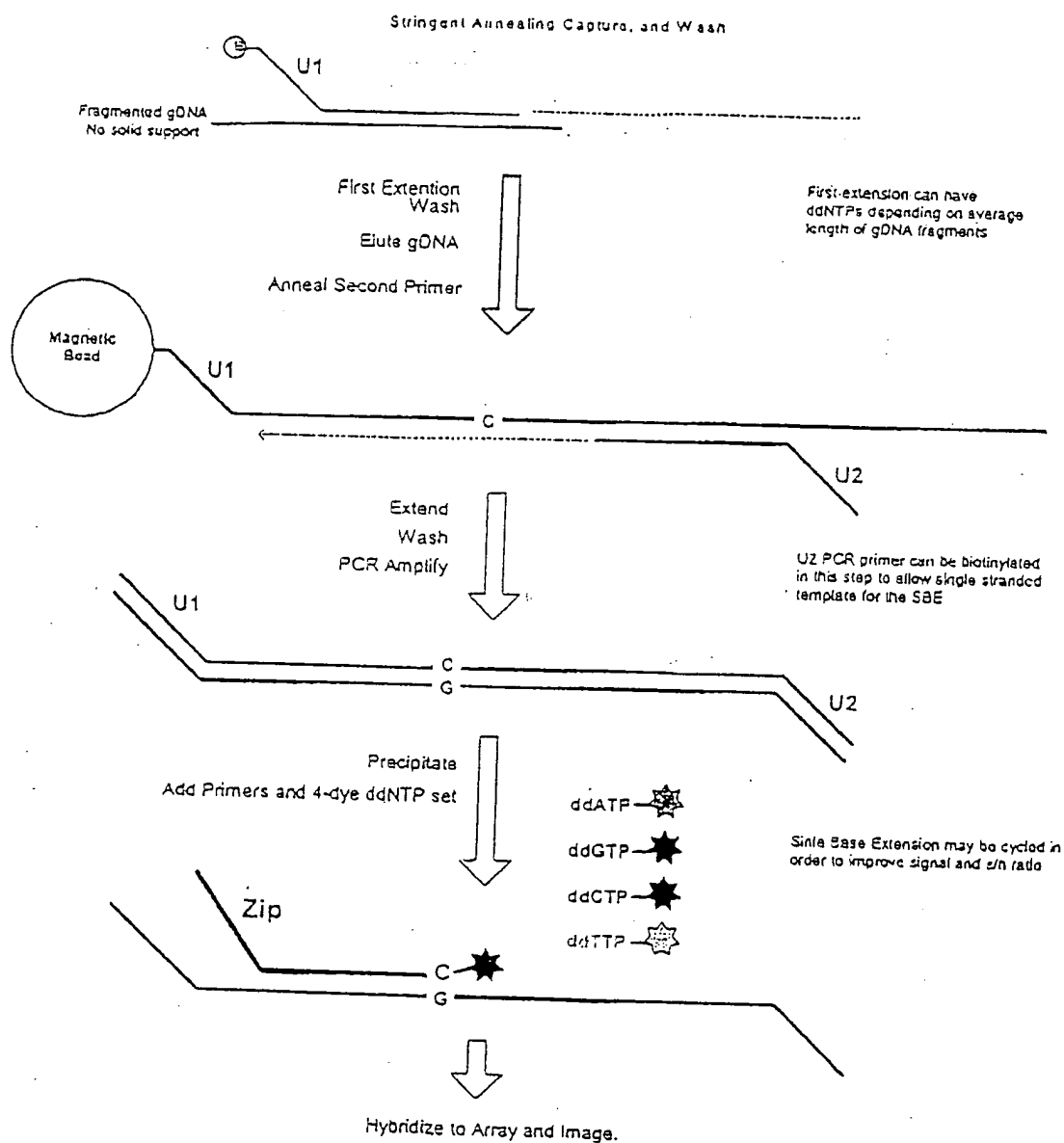
Reduced GenomeSingle Base Extension Assay:

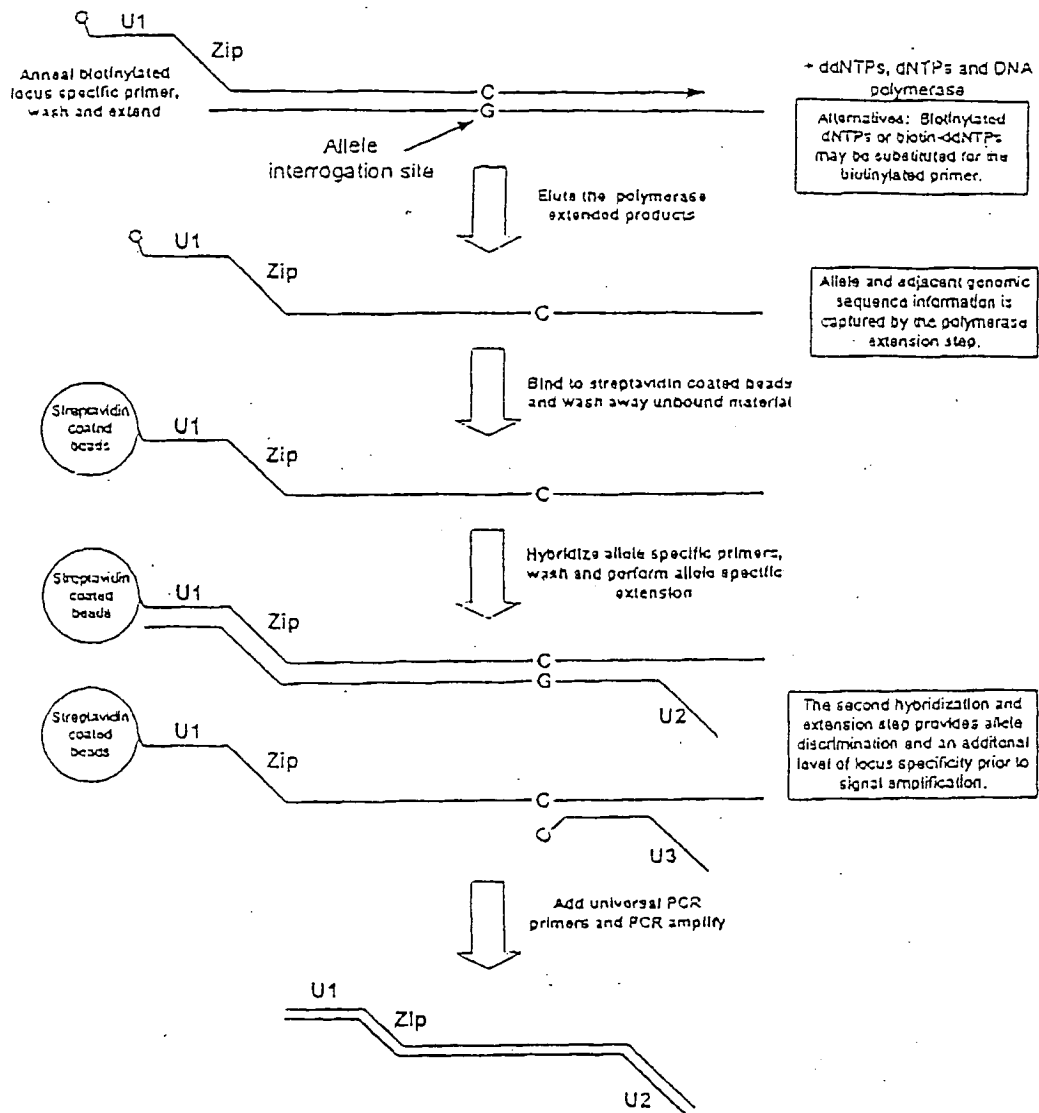FIGURE 2

Complexity Reduction and Multiplex Assay

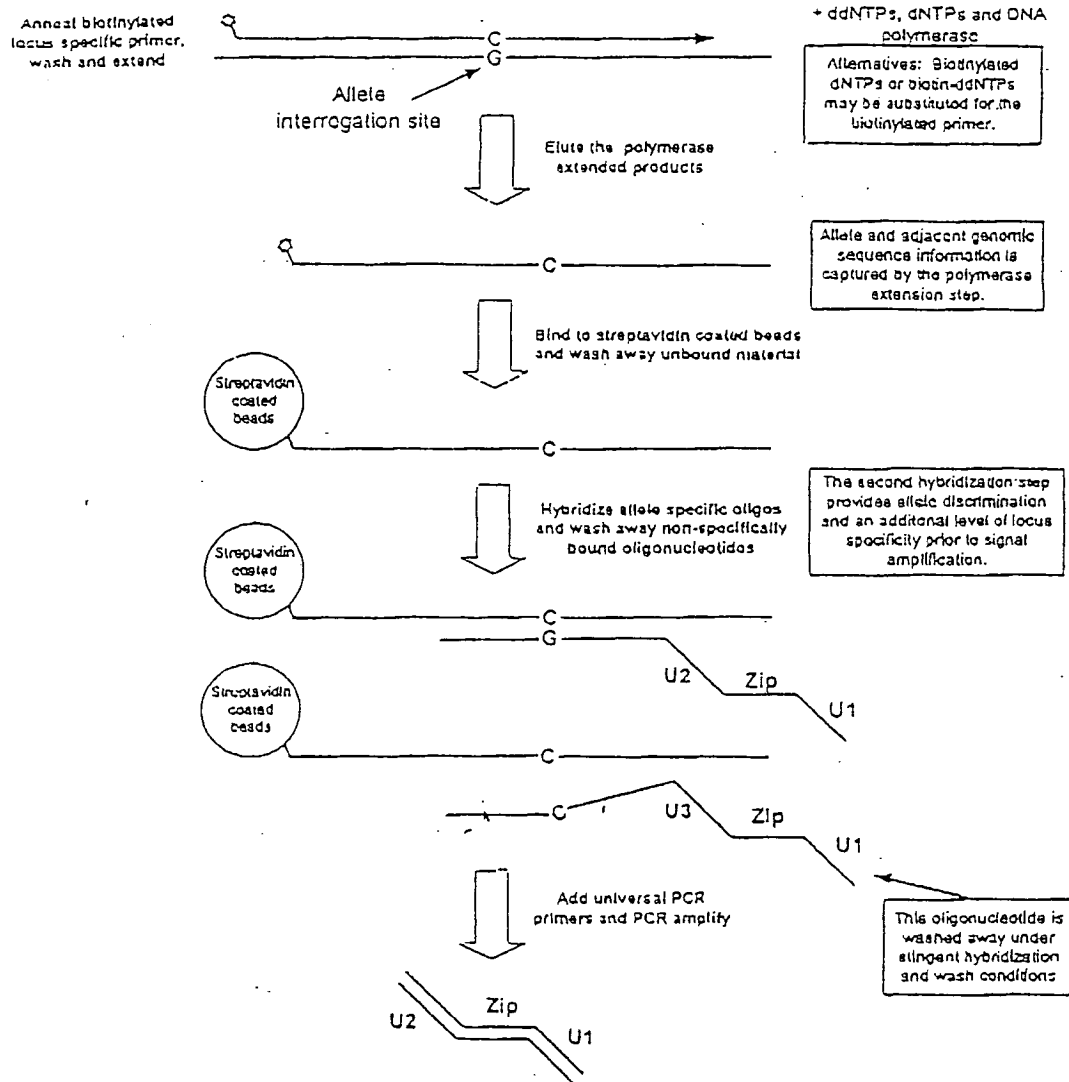FIGURE 3

Complexity Reduction and Multiplex Assay
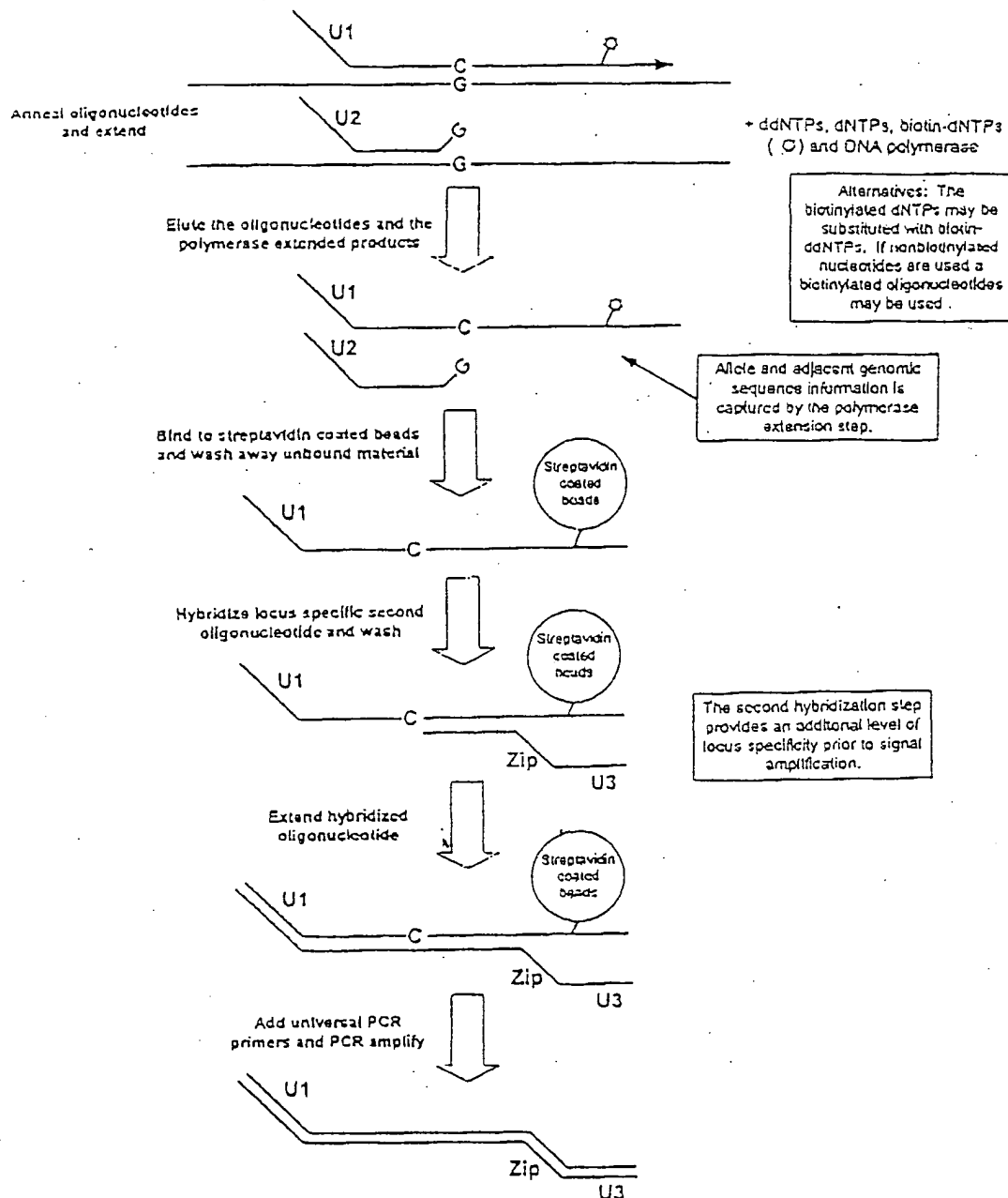

FIGURE 4

Complexity reduction and multiplex Assay
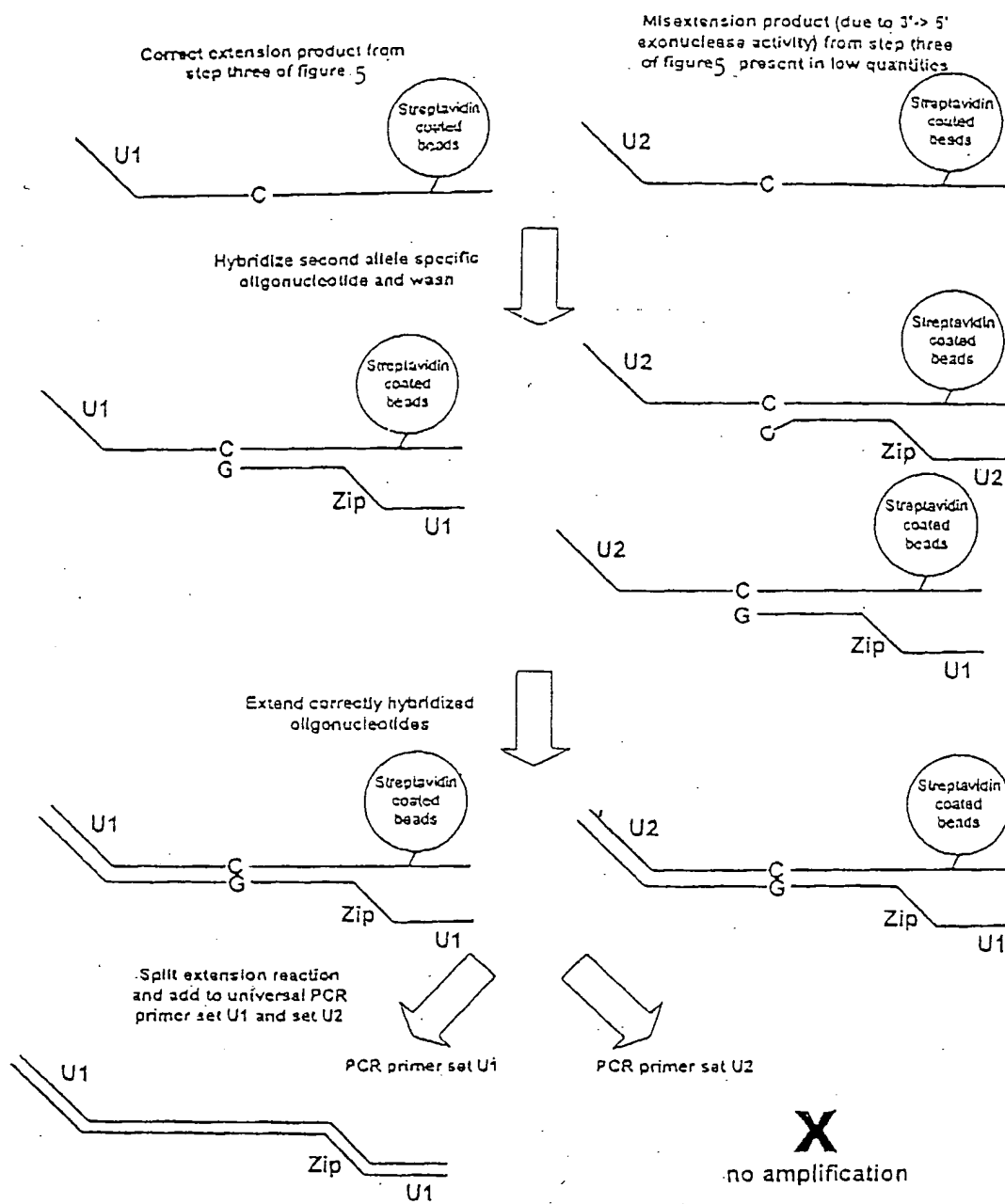

FIGURE 5

Complexity Reduction and Multiplex Assay

FIGURE 6

Solid Phase Locus-Specific Primer Extension

Starting material is immobilized, single stranded universal
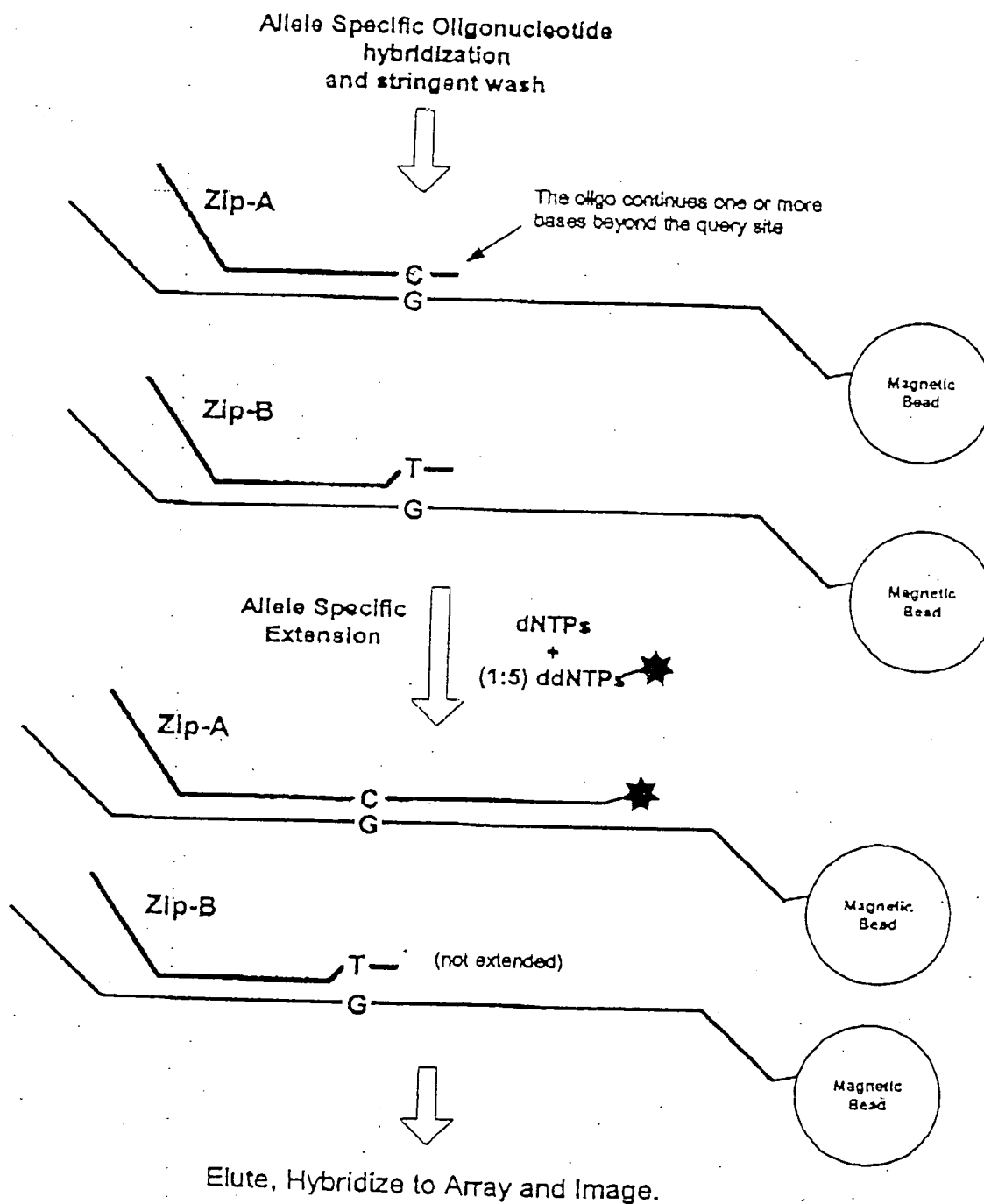PCR product. There are several ways to generate this.

FIGURE 7

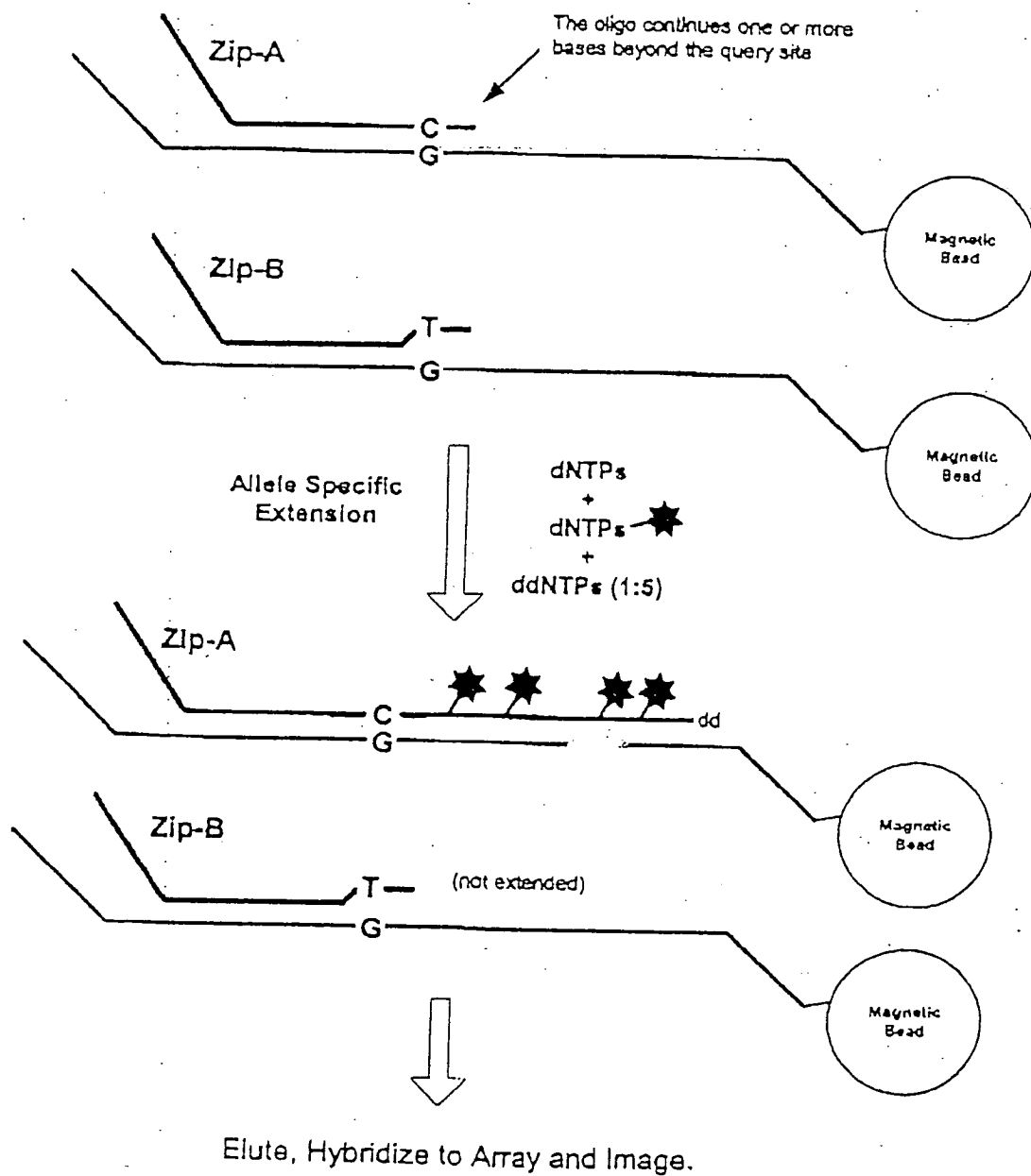Alternate labeling scheme for primer extension (high signal)
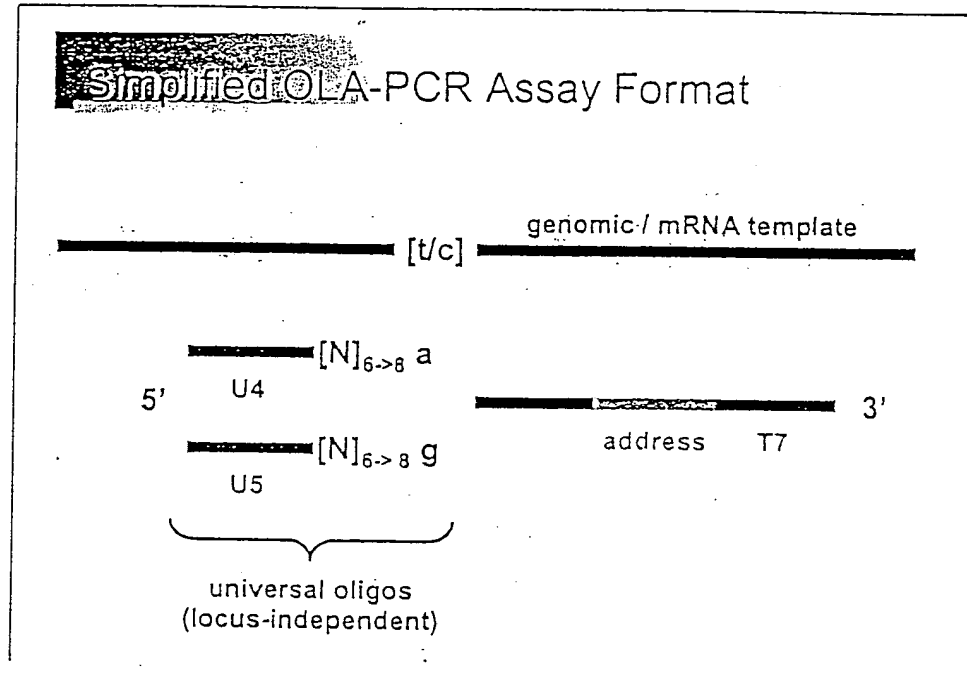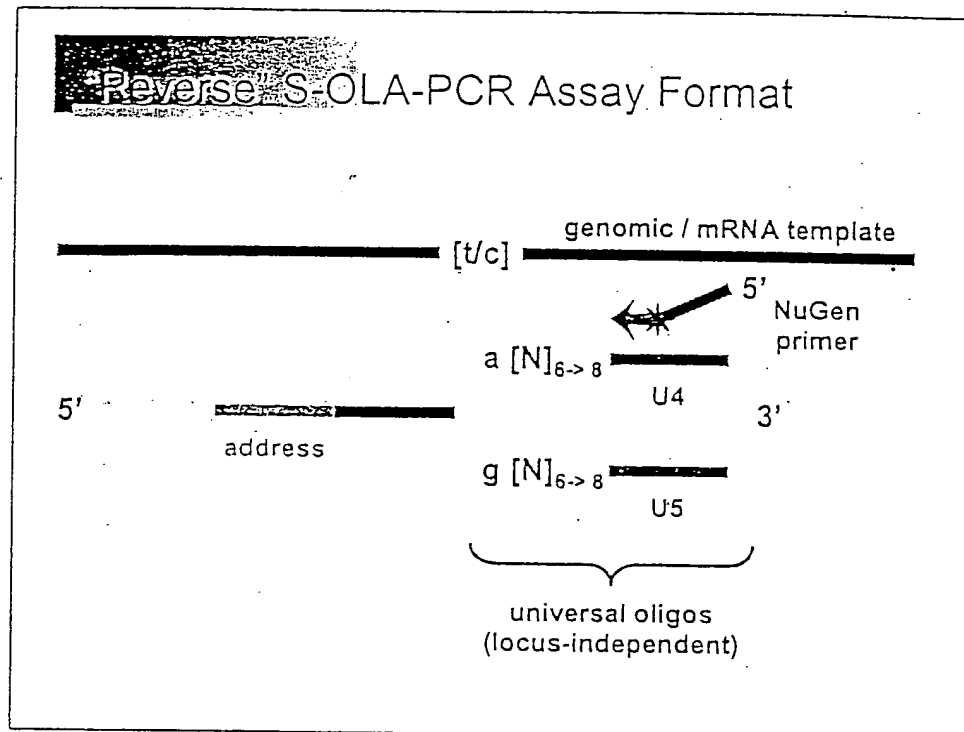

FIGURE 8

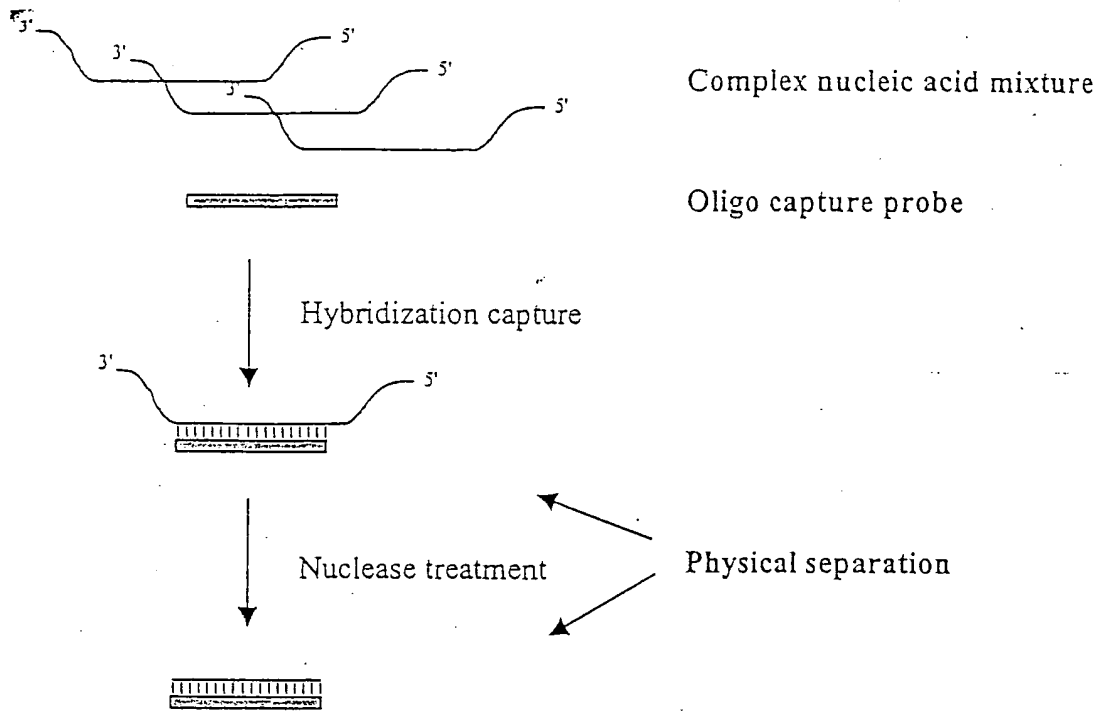# Simplified OLA-PCR Assay Format

genomic / mRNA template

━━━━━━━━━━━━━ [t/c] ━━━━━━━━━━━━━━━━━

━━━━━━[N]$_{6 \to 8}$ a

5'   U4   ━━━━━━━━━━━━━━━ 3'

━━━━━━[N]$_{6 \to 8}$ g   address   T7

U5

universal oligos
(locus-independent)

FIGURE 9

FIGURE 10

Complex nucleic acid mixture

Oligo capture probe

Hybridization capture

Nuclease treatment   Physical separation

FIGURE 11

Principle of ICAN method

FIGURE 12

Target

Primers

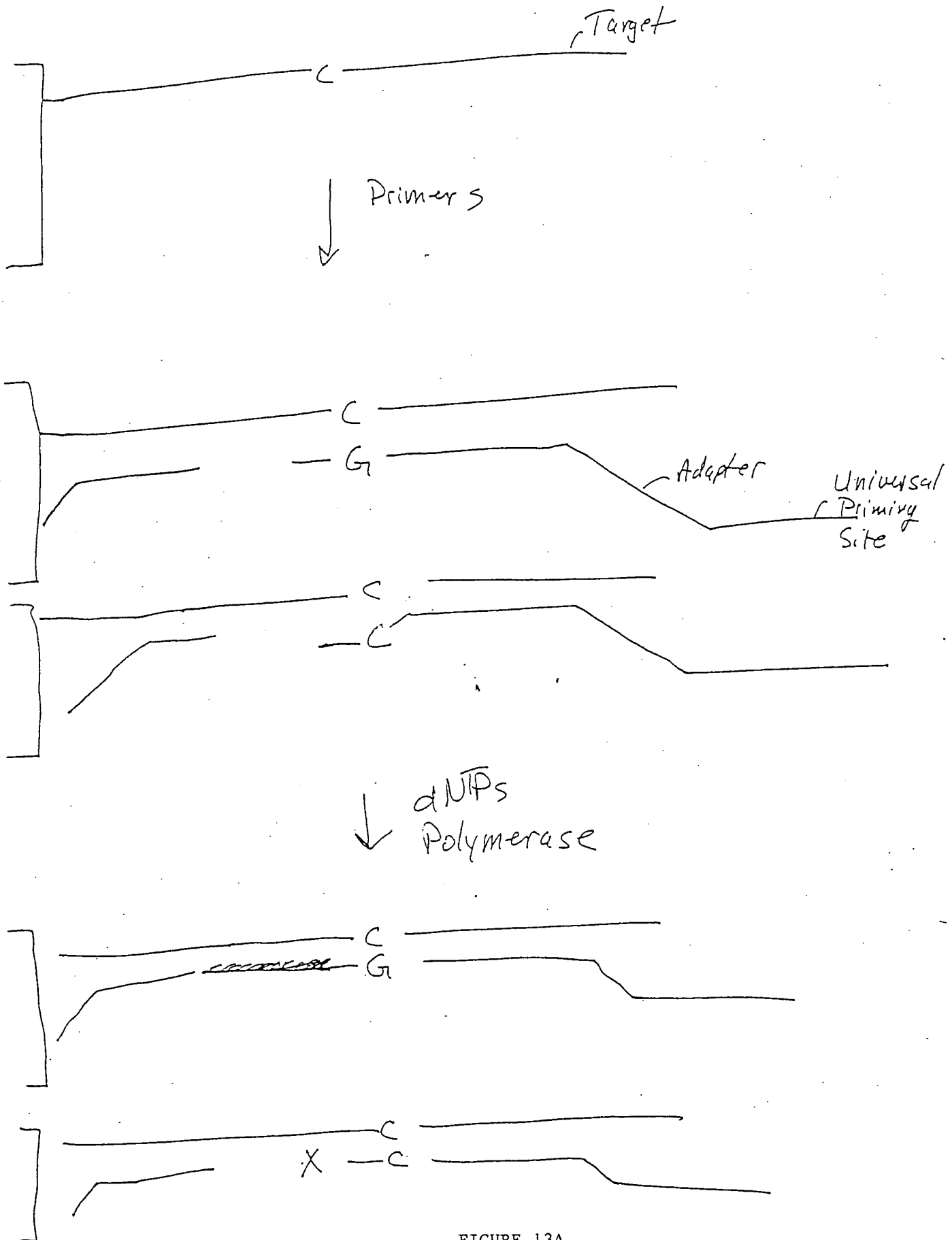Adapter

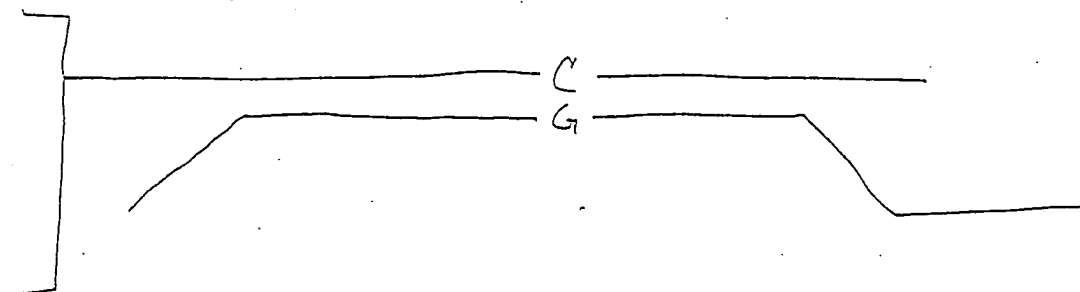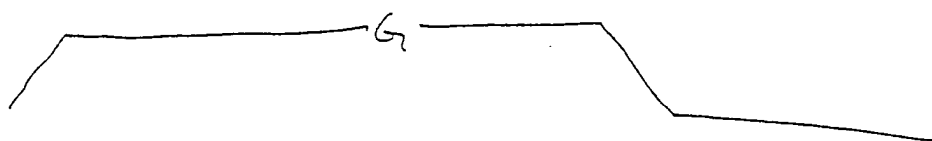Universal
Priming
Site

dNTPs
Polymerase

FIGURE 13A

↓ Ligase
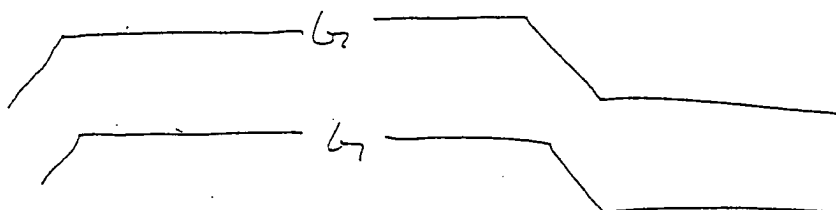
C

G

↓ Denature

G
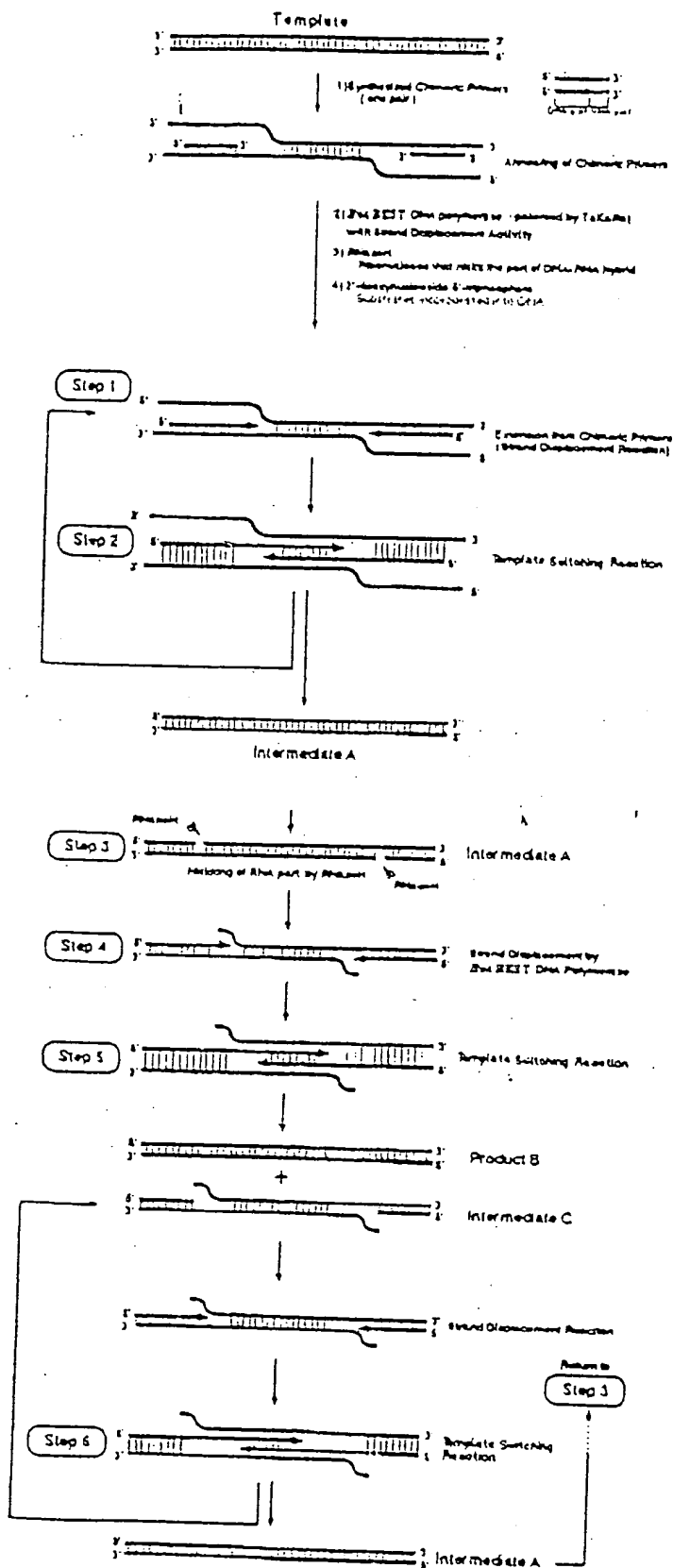
↓ + Primers
+ dNTP*
+ Amplification Enzyme

G

G

↓

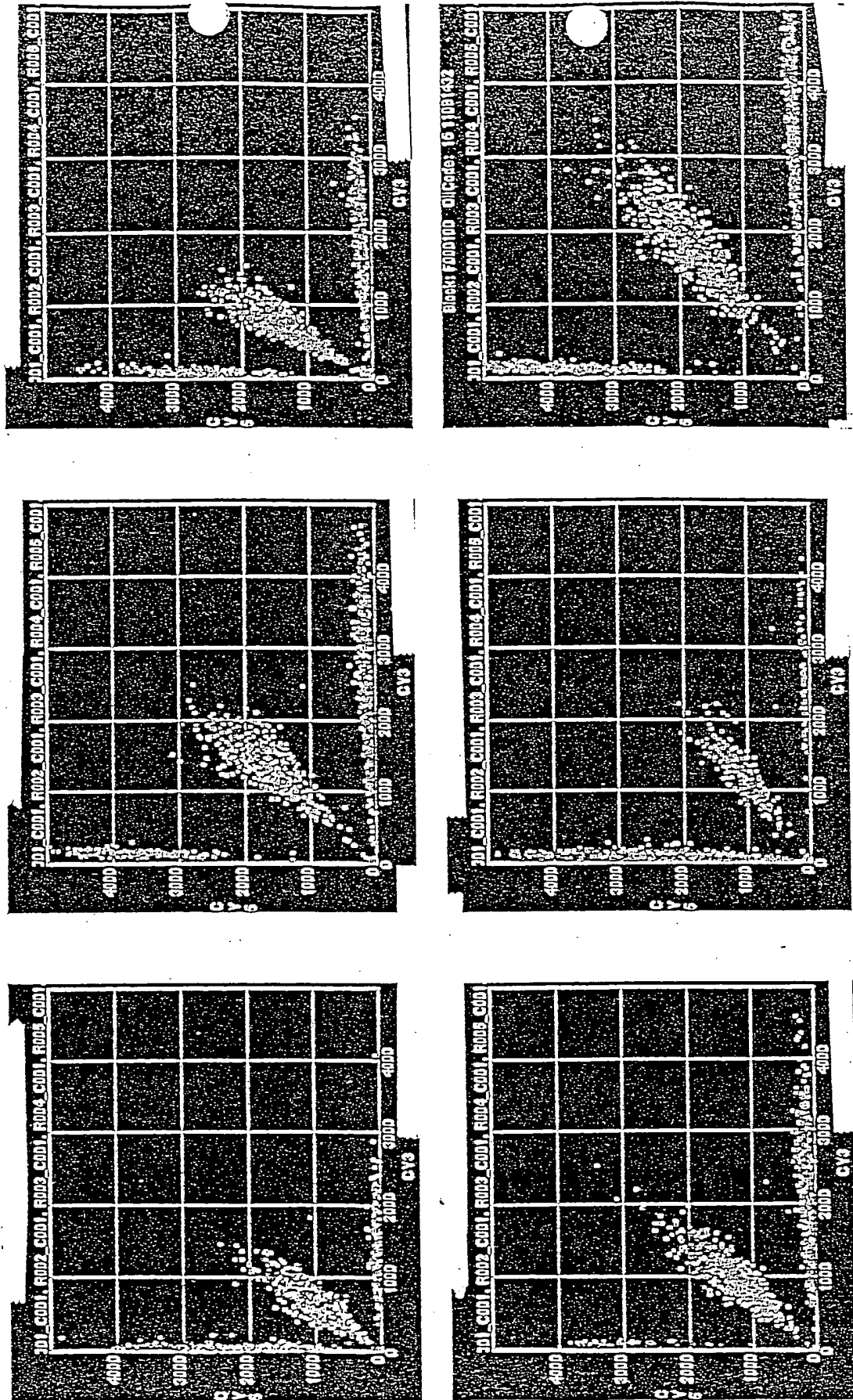Detect

FIGURE 13B

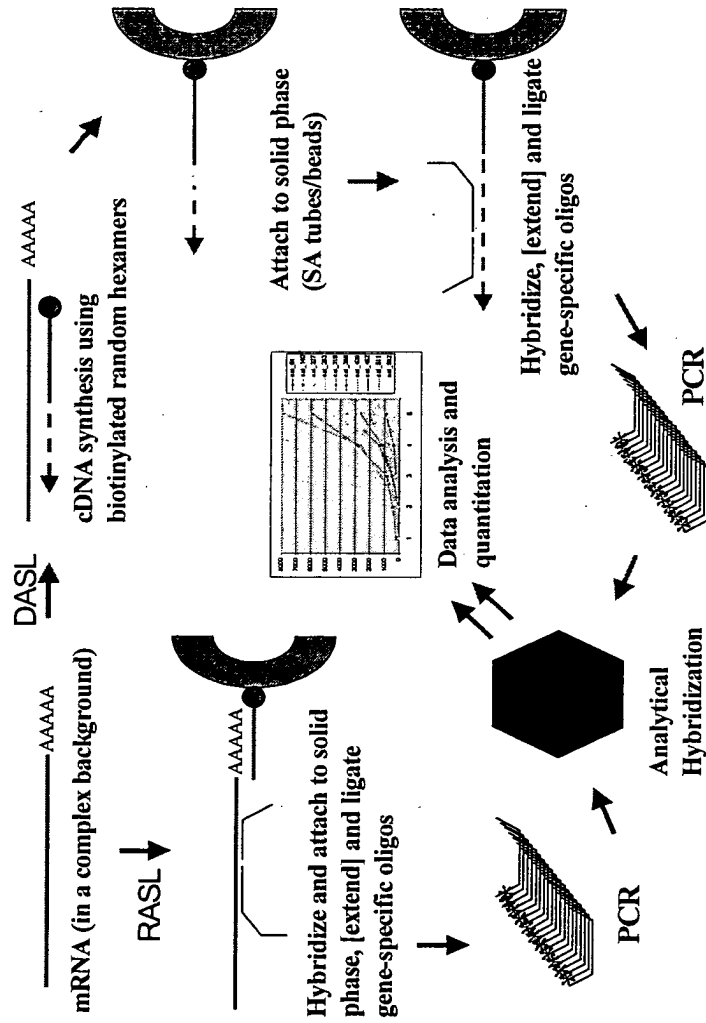SNP genotyping: 1152 Multiplex; 96 DNAs

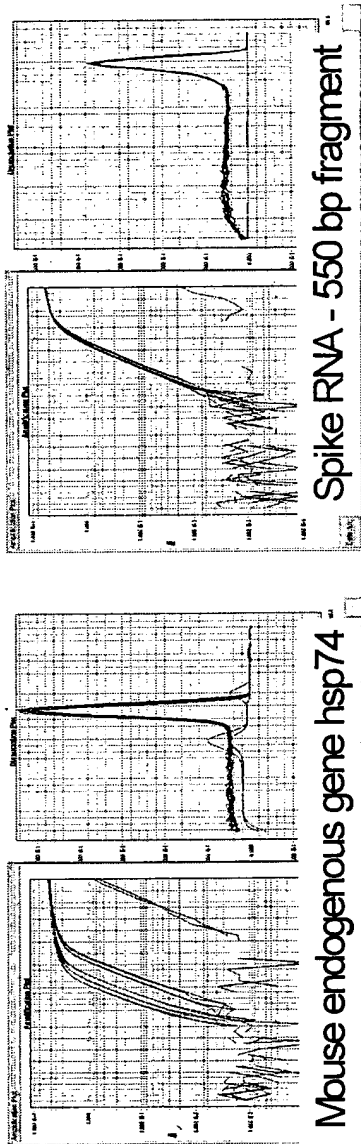FIGURE 14

FIGURE 15

FIGURE 16

		100 ng	500 ng
gfp 24	Avg	23.57	23.23
	SD	0.42	0.48
	CV	0.02	0.02
CG0675	Avg	19.78	17.20
	SD	0.66	0.15
	CV	0.03	0.01

Mouse endogenous gene hsp74

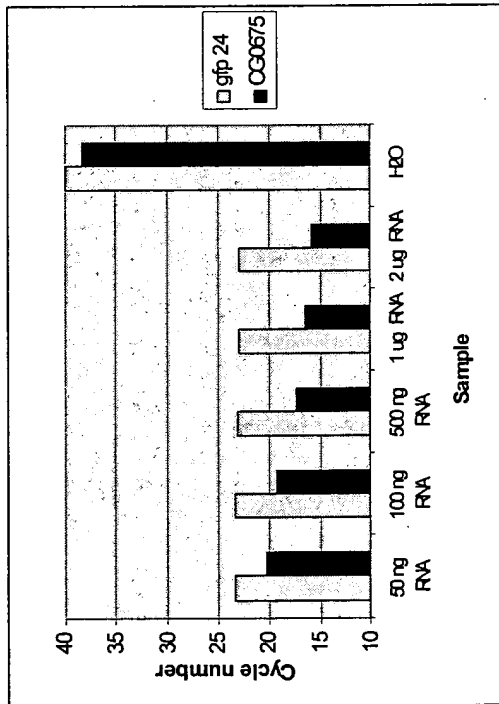- cDNA synthesis on the robot was reproducible and as good as in the manual reaction (at least for the tested genes).

FIGURE 17

|	pool 1	pool 2	pool 3	pool 4	pool 5	pool 6	pool 7	pool 8
cat	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07
cre	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00
E1A	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04
GFP	1.00E+05	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04
gus	3.00E+05	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05
lacZ	1.00E+06	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05
luc	3.00E+06	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06
neo	1.00E+07	0.00E+00	1.00E+04	3.00E+04	1.00E+05	3.00E+05	1.00E+06	3.00E+06
bla	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05
GST	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05	3.00E+05

FIGURE 18

Selecting 3 probes that perform well gives better data than averaging all probes.
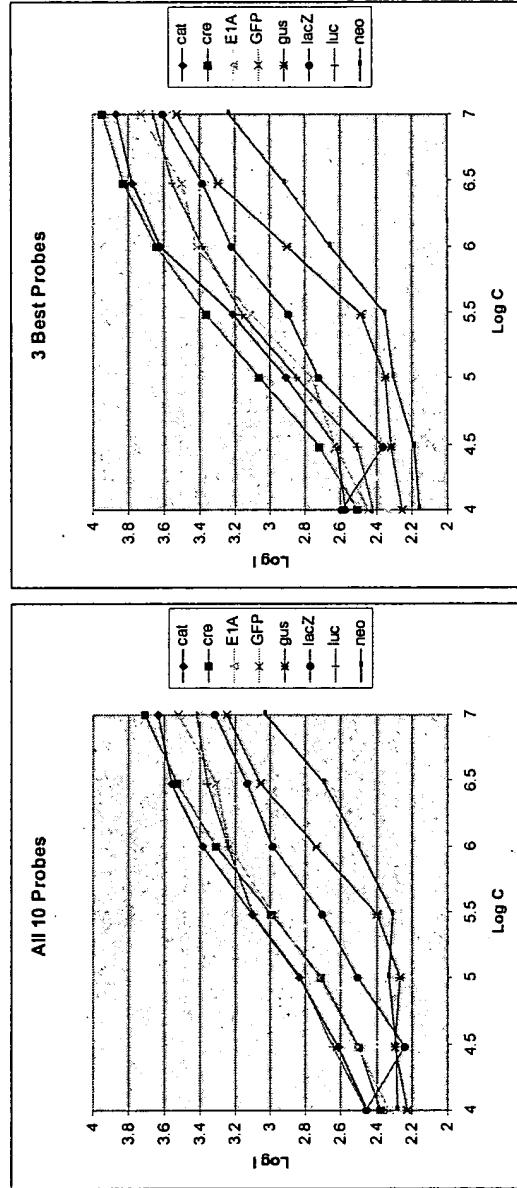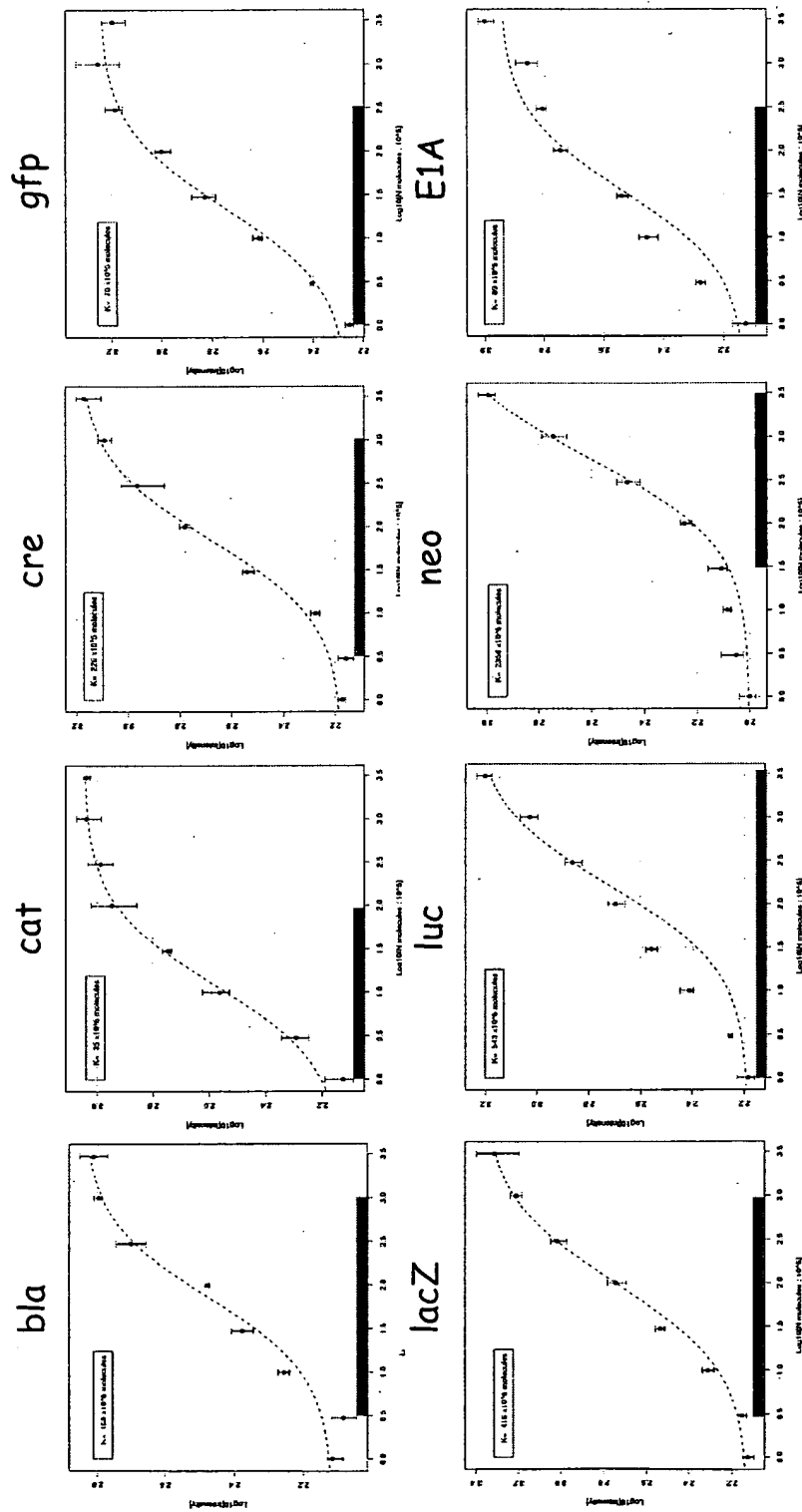

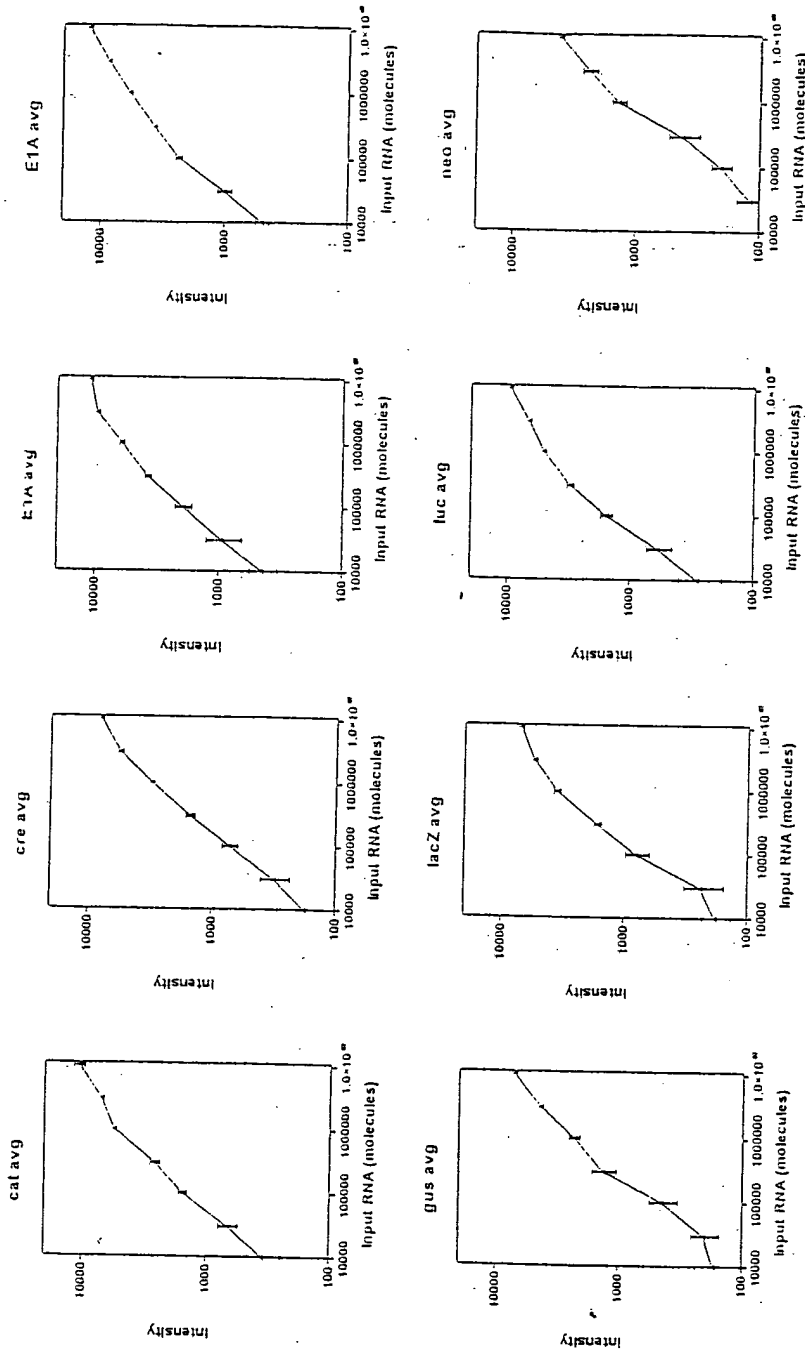Matrix 4, 238-plex, 100 ng total RNA background

FIGURE 19
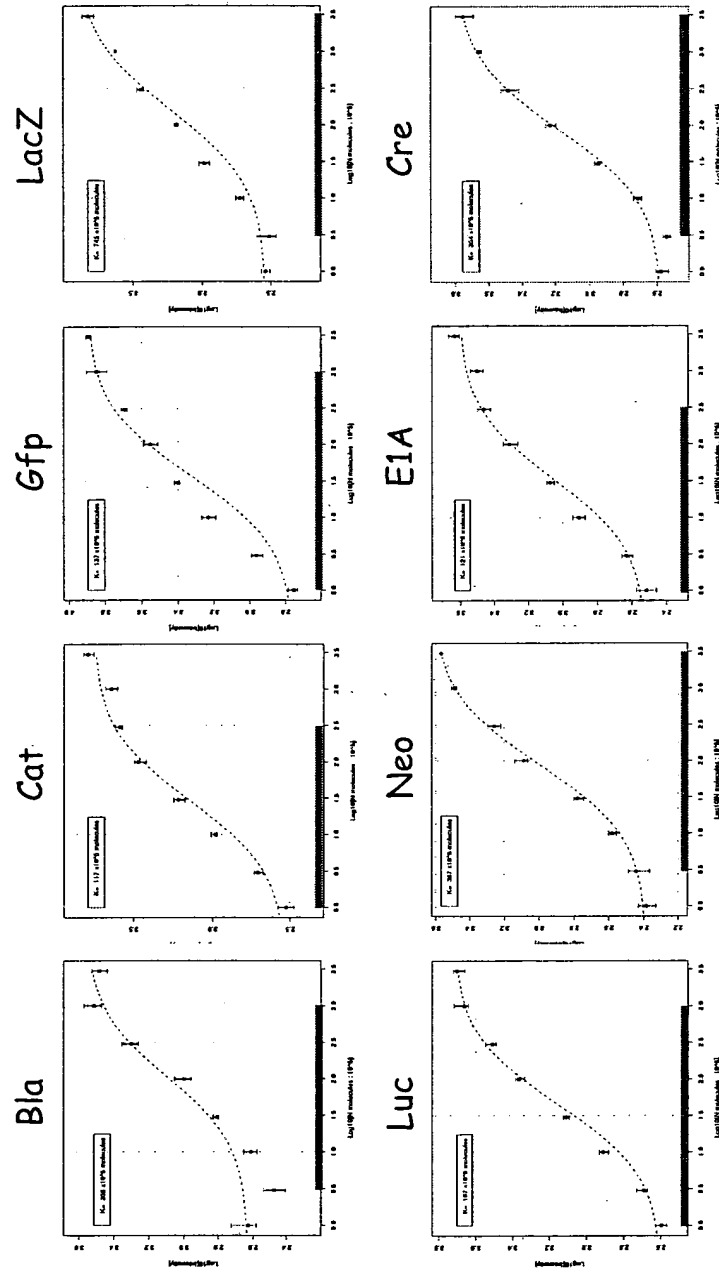
FIGURE 20

■ 3 fold detection range

●Error bars represent the range of intensities of 4 replicates.

250 ng of total RNA / sample
Ds DNA hybridization
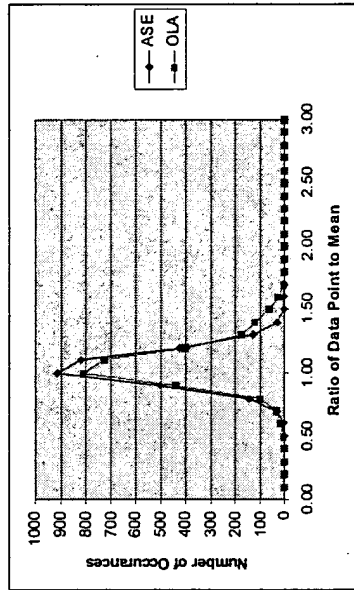Error bars represent the range of intensities of 4 replicates.

FIGURE 21

※100 ng total RNA background, 12 replicates, 238-plex.

※all pre-PCR and post-PCR processes identical to SciOps
including single stranded product hybridization to arrays.

※Dynamic range: 2.5 – 3 logs; Precision: better than 3 fold change.

FIGURE 22

(500 ng input RNA)

● 100.0% data points among 4 replicates within 2 fold change
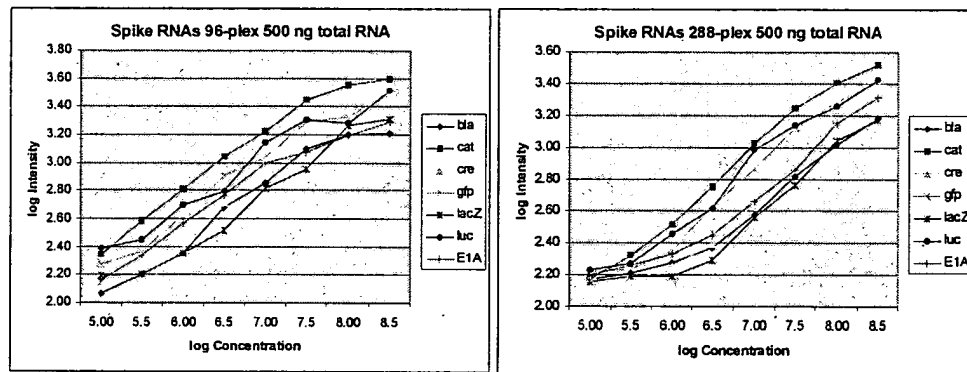◉ 99.8% data points among 4 replicates within 2 fold change
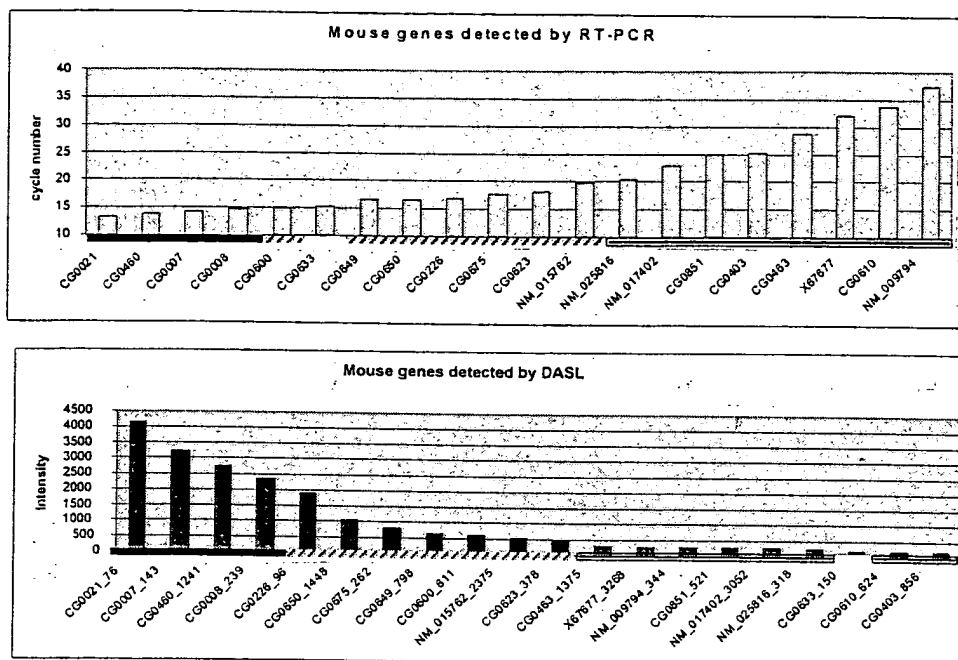
FIGURE 23

FIGURE 24

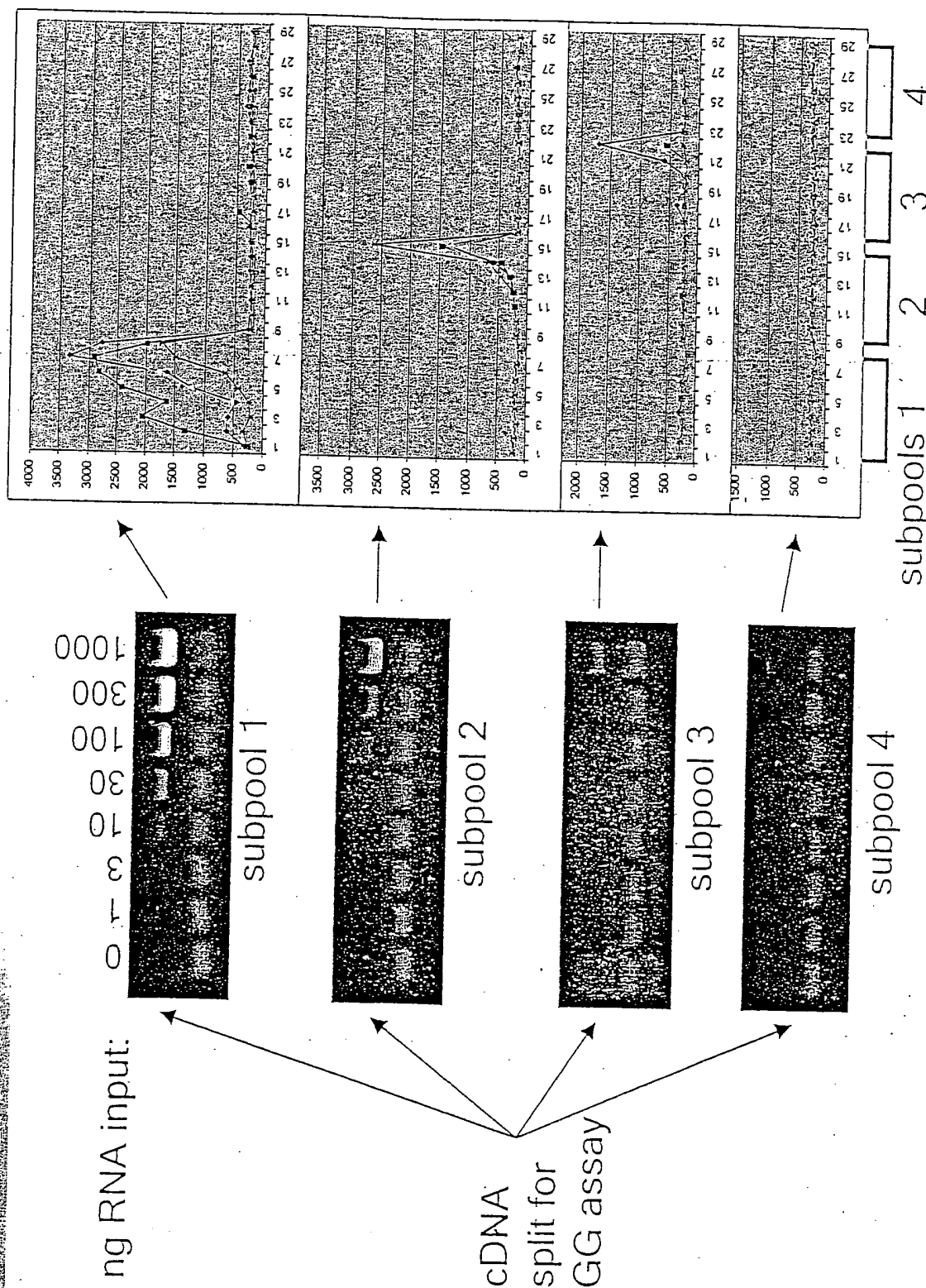FIGURE 25

How to Handle Genes Expressed at Different Levels?

FIGURE 26
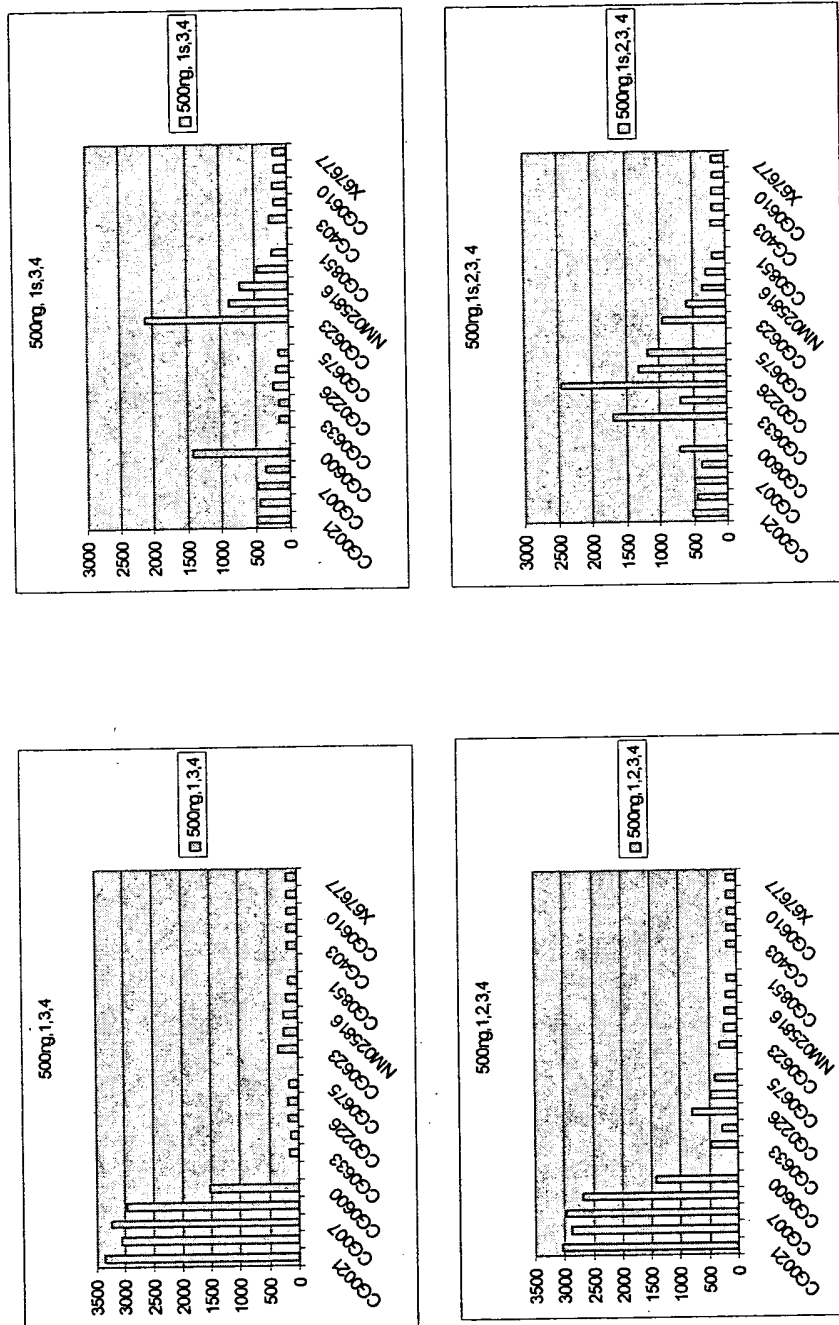
FIGURE 27